Taking the brakes off the immune system: modelling effects in non-clinical safety studies

Dr Kirsty Harper, Head of Biologics, UK
Overview

- Introduction to the immune system
- Cancer and the immune system
- Checkpoint inhibitor targets and associated biology
- Autoimmunity – causes and presentation
- Strategies for effective non-clinical safety assessment of CPI products
- Summary
Introduction to the immune system
Function of the immune system

+ The main role of the immune system is to protect the body from infection
  + Variety of cell types and systems in place to effectively identify and remove pathogens before they can cause damage to the immune system
The immune system – key components

- CD8+ T cell
- CD4+ T cell
- B cell
- Monocyte
- Eosinophil
- Basophil
- Neutrophil
- Dendritic cell
- Erythrocyte
- Platelets
- Macrophage
- T helper 1
- T helper 2
- T helper 17 etc
- Plasma cell
- NK cell
- Common myeloid progenitor
- Pluripotent Stem cell
- Common lymphoid progenitor
- Granulocytes

**Legend:**
- **Red pathway:** Erythrocyte → Monocyte → Macrophage → Eosinophil, Basophil, Neutrophil
- **Green pathway:** Common myeloid progenitor → Monocyte → Macrophage → Eosinophil, Basophil, Neutrophil
- **Blue pathway:** Common lymphoid progenitor → NK cell → CD8+ T cell, CD4+ T cell, T helper 1, T helper 2, T helper 17 etc
The main role of the immune system is to protect the body from infection.

- Variety of cell types and systems in place to effectively identify and remove pathogens before they can cause damage to the immune system.

Two main arms of the immune system:

- Innate immune system
- Adaptive immune system
The innate immune system

+ Innate responses are the foundation of immunity
  + All organisms, even bacteria, have innate defense mechanisms
  + Ancient systems, some level of conservation with lower order species

+ Combination of cell populations and soluble factors which work to damage and remove pathogens
  + Complement, TLRs, granulocyte populations, type I interferons and other cytokines
  + First wave of immune attack; can cause collateral damage e.g. temperature increase, cell death
  + Predominantly utilises pattern recognition to identify foreign material
The innate immune system

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- CD4+ T cell
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- Macrophage
- Monocyte
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- CD4+ T cell
- NK cell
The toll like receptor system

Limitations of innate immunity

+ Parasitic agents grow more quickly than innate immunity can control
+ There is only limited amplification of the response
+ There is no memory so the next time you encounter the same pathogen the response time is the same
+ Higher organisms have evolved an additional, more sophisticated immune system – the adaptive immune system
  + Some crossover between the two systems with some cell populations being important for bridging from the initial innate response to a subsequent adaptive response
The adaptive immune system

- Second wave of immune attack
  - Includes cells which specifically target the pathogen to minimise collateral damage
    - Provides a mechanism for immune recognition that can evolve as rapidly as the parasite (clonal selection)
    - There is rapid amplification of a response
- Key aspect of the adaptive arm of the response is memory
- Specific cell populations are retained after clearance of a pathogen which are uniquely primed to remember and attack that pathogen upon further encounter
  - Future exposure to the same pathogen is subclinical due to rapid, specific recognition and clearance
- Two arms of the adaptive system: cellular and humoral
The adaptive immune response

- Common myeloid progenitor
- Pluripotent Stem cell
- Common lymphoid progenitor

- Erythrocyte
- Platelets
- Monocyte
- Macrophage
- Dendritic cell
- Neutrophil
- Eosinophil
- Basophil
- Granulocytes
- NK cell
- CD8+ T cell
- CD4+ T cell
- Plasma cell
- T helper 1
- T helper 2
- T helper 17 etc
Tolerance
Tolerance

+ One aspect critical for an effective and controlled immune system
  + Identification of self vs non-self
+ Mediated via tolerance mechanisms
+ Central tolerance
  + Selection of T and B cells meeting specific criteria, mediated by specific antigen presenting cell populations in the thymus or bone marrow.
  + Leads to deletion of cells that are under or over-active
  + Ensures that only cells with appropriate responses to self and non-self antigens will be released into the periphery
  + Recognition of non-self antigens to mediate adequate surveillance
  + Limited responses to self antigens to prevent damage of organs and tissues
Central tolerance

Positive selection

- Immature T cell
- Recognition of MHC
- No recognition of MHC
- Death by neglect

Negative selection

- Thymic epithelial cell
- Strong response to self antigen
- Death by apoptosis

Exit to periphery

- Recognition of MHC
- Weak response to self antigen
Peripheral tolerance

+ Central tolerance is effective but not perfect
+ Additional control mechanisms exist in lymphoid organs to further refine responses to self and non-self antigens
  + Limits collateral damage and ensures that immune system reverts to baseline once the pathogen is removed
+ Multiple methods by which potentially damaging responses are controlled
+ Exogenous control mechanisms
  + Regulatory T cells
  + Inhibitory cytokines
  + Direct tolerance induction vs bystander tolerance
+ Endogenous control mechanisms
  + Induction of anergy or exhaustion
For effective immune function, it is critical that there is a BALANCE between identifying pathogens and controlling subsequent responses.

Identification and removal of pathogens
Identification of tumour cells
Controlled and self-limiting responses
For effective immune function, it is critical that there is a BALANCE between identifying pathogens and not over-reacting/damaging self tissues.

Immune system balance

- Excessive stimulation
- Hypersensitivity
- Autoimmunity

Excessive stimulation
For effective immune function, it is critical that there is a BALANCE between identifying pathogens and not over-reacting/damaging self tissues.

- Increased risk of infection
- Decreased tumour surveillance

Immunosuppression
Cancer and the immune system
Cancer and the immune system

+ Tumours actively seek to avoid detection by the immune system
  + Decrease expression of markers that make them visible to the immune system
  + Utilise control mechanisms to dampen immune responses locally
    + Expression of inhibitory markers
    + Production of suppressive cytokines
    + Generation of cell populations with inhibitory properties
    + Induction of anergy or exhaustion in effector T cell populations
+ Results in a lack of prolonged or efficient tumour killing
+ Major focus in immunotherapy is to increase tumour surveillance by preventing this evasion
Immunomodulatory therapeutics in development

Clearing antibodies
T cells
NK cells
NK T cells
Immunostimulatory antibodies
DNA vaccines
Peptide vaccines
Modified tumour cell vaccines
Dendritic cell vaccines
Cytokines
Current state of cancer immunotherapy

- There are multiple different platforms of cancer immunotherapy and many very different target pathways
- Adoptive cell transfer
  - TILs, TCR–transfected T-cell, CAR therapy
- Non-specific immunotherapies
  - Cytokines (IL-2, IFNα) CD40 agonist mAb, TLR agonist
- Vaccination strategy
  - Sipuleucel-T, MAGE-3 ASCL, OncoVEX
- Immune checkpoint blockade
  - CTLA-4 blocking mAb, PD-1 blocking mAb
- The remainder of this talk with focus upon this latter approach so called immune checkpoint targeting antibodies
Inhibition of check point targets
T-cell killing of tumour cells can be switched on and off
  - Regulated by signals from other cells of the immune system such as antigen presenting cells and tumours themselves

T-cells are effectively “switched off” due to signalling via immunoinhibitory (checkpoint) receptors on activated T cells

T cells can be switched back on in lymph nodes or the tumour microenvironment by drugs that target checkpoint receptors

There are many such drugs in development and on the market today with some exciting biology behind them
  - PD1, CTLA-4, PDL, KIR, IDO1, 4-1BB, OX40, LAG3, B7-H3, CD27, CD70, CD28, CD30 etc.
Checkpoint inhibitors: background
Potential checkpoint targets (not exhaustive)

Activating receptors
+ CD28
+ OX40
+ GITR
+ CD137
+ CD27
+ HVEM

Inhibitory receptors
+ CTLA-4
+ PD-1
+ TIM-3
+ BTLA
+ VISTA
+ LAG-3

Checkpoint inhibitors: Mechanism of action

+ Immunoinhibitory receptors are expressed after normal MHC class I/II adaptive T cell activation
+ Ligation of CTLA-4 by B7-1/B7-2 or PD1 by PD-L1 or PD-L2 attenuates normal T cell activation
+ CTLA-4 and PD1 upregulation on chronically activated T cells makes them less responsive to antigenic stimulation
+ Inhibition of these targets can restore T cell function and enhance T-cell tumour killing
+ Checkpoint inhibitors play critical roles in maintenance of peripheral T cell tolerance
+ Drugs that target immune system checkpoints have the potential to promote inflammation/autoimmunity in humans
Autoimmune disease
The immune system operates at a balance. Too much stimulation can induce autoimmune disease.
Autoimmune disease

+ Caused by a breakdown in tolerance, where self-antigens are recognised as immune targets
+ Can present as systemic disorders or localised disease
  + Systemic autoimmune diseases: SLE, Sjogren’s syndrome, RA
  + Localised autoimmune diseases: T1D, Hashimoto’s thyroiditis, MS
+ Multiple mechanisms implicated in the induction of autoimmune disease
  + Escape of self-reactive T cells into the periphery
  + Loss of or non-functional regulatory T cells
  + Molecular mimicry
  + **Loss of control mechanisms to limit immune responses**
+ Leads to inflammation and destruction of the target tissue
  + Killing of pancreatic islet beta cells in T1D
  + Destruction of the myelin sheath in MS
Checkpoint inhibitors have the potential to induce AI due to removal of part of the control mechanism of the immune response

- Mouse models lacking key check point markers demonstrate autoimmune disease
CTLA-4

- Cytotoxic T lymphocyte antigen
- Expressed constitutively by naturally occurring FoxP3+ regulatory T cells or following activation of other T cell populations
- Binds with high affinity to CD80 and CD86 on APCs, out competing binding of CD28 to the same ligands
- Binding through CTLA-4 sends an inhibitory signal to the cell

CTLA-4 deficiency leads to severe autoimmune disease in a mouse model

- T cell lymphoproliferative disorder marked by splenomegaly, lymphadenopathy, reduced growth poor survival
- Circulating T cells characterised by an activated phenotype
- Cause of death thought to be autoimmune-mediated cardiac failure

Tai et al. (2007). Proc Natl Acad Sci USA 104(34):13756-13761
**PD-1 and PD-L1**
- Programmed cell death protein 1 and programmed cell death protein ligand 1
- PD-1: Expressed on activated T cells, B cells and NK cells
- PD-L1: upregulated on activated T cells and APCs, expressed on tumour cells

**PD-1 knock out mice**
- Strain-specific autoimmunity presenting later in life
- Enhanced proliferation of CD8⁺ T cells and increased cytotoxicity

**PD-L1 knock out mice**
- Enhanced APC-mediated activation of T cells
- Increased CD4⁺ and CD8⁺ T cell activation and function
- Increased susceptibility to autoimmune disease
The differing phenotypes of checkpoint-deficient models illustrate the redundancy of the immune system

- Multiple mechanisms of control
- Each checkpoint marker has a specific pattern of expression resulting in varying effects when eliminated from the immune system
- The immune system may still be able to cope with the elimination of one control mechanism, but there is potential for additive effects when multiple pathways are blocked
Checkpoint inhibitor products in the clinic

+ Varying response, some patients seeing dramatic effects
  + Both CTLA-4 and PD-1 blockade significantly increased patient survival in Ph III melanoma trials
  + No efficacy observed by blocking CTLA-4 signalling in a Ph III NSLC clinical trial
  + Suggests that the response to treatment may be affected by additional factors; starting to look at stratifying patients and combination of CPI products

+ Immune related AEs observed in patients
  + 77% of patients receiving Ipilimumab, 38% of which were severe and included elevated liver enzymes, hepatitis and enterocolitis
  + Treatment with glucocorticoids required to manage effects

+ Increased cause for concern?
  + Already removing control mechanism in studies utilising a single checkpoint inhibitor, will there be additive effects when knocking out multiple pathways?
  + For effective safety assessment it is critical to understand the effect of altering these signalling pathways, both individually and in combination
Non-clinical safety assessment strategies for CPI products
Challenges in safety assessment

- The intended pharmacological action of each therapeutic is immune system activation – immediate alarm bells!
- As with most biopharmaceuticals the balance between therapeutically desired pharmacology and clinical dose-limiting “exaggerated pharmacology” is a delicate balance
- Acute effects such as systemic cytokine release may be manageable in some clinical situations
- More chronic effects such as induction of autoimmune disease may be more difficult to model in healthy standard non-clinical models
- SPF animals may also lack or have limited target expression of checkpoint molecules
Nonclinical safety assessment strategies to assess CPI products

+ As described in ICH S6 (R1) these products must be assessed in pharmacologically relevant species
+ BUT the required considerations go beyond just confirming that the product can bind the target and induce relevant pharmacology
  + Must ensure that the immune status in non-clinical species vs the human population is understood
  + Density of target expression
  + Extent of antigen experience
  + Effects of chemotherapy???
Yervoy (Ipilimumab) anti-CTLA-4 antibody

+ Binds human CTLA-4 \textit{in vitro} with high affinity
+ Inhibits \textit{in vitro} binding of B7.1 (CD80) and B7.2 (CD86) to human CTLA-4
+ CDC and ADCC activity investigated \textit{in vitro} and \textit{in vivo}
+ Colon carcinoma studies in human CTLA-4 transgenic mouse
+ Extensive TDAR investigation in cynomolgus monkey
  + Hepatitis B surface antigen (HBsAg) vaccine, a melanoma cell-based vaccine (Sk-mel), DNP (2,4-Dinitrophenyl)-Ficoll, keyhole limpet hemocyanin (KLH) and simian immunodeficiency virus (SIV), DNA vaccines (purified plasmid DNA) expressing the proteins for the gag (pSIVgag), env (pSIVenv), and pol (pSIVpol) portions of SIV. In one study, the SKmel tumour line was transfected to express GM-CSF.
  + Extensive immunophenotypic analysis of T cells and subsets in these studies
  + Extensive assessment of humoral immune responses to TDAR antigens
Yervoy (Ipilimumab) anti-CTLA-4 antibody

+ Tissue cross reactivity studies
+ Safety pharmacology included as part of repeat dose toxicity programme
+ Combination studies with anti-CD137 and anti-PD-1 antibodies conducted in cynomolgus monkeys
+ Single species toxicology programme conducted in cynomolgus monkey as only pharmacologically relevant species
+ Studies up to 6 months in duration at a variety of dose levels conducted
+ No reproductive, developmental or juvenile toxicology studies were submitted for registration purposes
Yervoy (Ipilimumab) anti-CTLA-4 antibody

+ Most findings considered adverse seen in combination toxicity or exploratory (TDAR) pharmacology studies not anti-CTLA-4 alone GLP studies
+ Low incidence of immune-mediated toxicities
  + Colitis, dermatitis, or infusion reactions
  + Responses consistent with proposed MoA of CTLA-4 in maintaining self-tolerance
+ Findings in cynomolgus monkeys correlated with findings in humans, although with less frequency – under prediction?
+ CTLA-4 blocking did result in an over stimulation of the T-cell compartment
  + Few meaningful changes in immunophenotype or autoimmune organ pathology (with exception of colitis and dermatitis)
  + Increased TDAR was observed demonstrating expected pharmacodynamics
CPIs in the clinic – side effects

+ As anticipated based on mode of action, CPI products are inducing immune-mediated adverse events in the clinic
  + 77% of patients receiving Ipilimumab, 38% of which were severe and included elevated liver enzymes, hepatitis and enterocolitis
  + Treatment with glucocorticoids required to manage effects

+ Evidence that AI findings are occurring, therefore need to be aware of AI and try to characterise the risk in non-clinical packages wherever possible
Non-clinical approaches for CPIs

+ Be mindful of the fact that the non-clinical safety package may be very clean
  + Assessment in animals with relatively naïve immune systems
  + No concurrent immune activation to increase the need for peripheral tolerance mediated via CP markers
  + From previous products, know that there are likely to be AI consequences in the clinic
    + Ideally want to generate data that will characterise the extent of any AI that will be induced in the clinic

+ Consider activation of the immune response within safety assessment studies
  + Ideally want to understand the effects of treatment the immune system in clinical population
  + Inclusion of FUNCTIONAL assessments of the immune system

+ Assess key tissues and organs for AI effects
Example of FIH-enabling programme for a CPI-targeting mAb

+ **TXR studies**
  + Human tissue, plus non-clinical species if additional information is required

+ **PK study**

+ **Preliminary and repeat dose toxicity studies in pharmacologically relevant species**
  + Including assessment of immune function as well as proportions of key populations
  + Selection of assays based on the specific mechanism of action
  + Assess those populations specifically affected by treatment if possible

+ **Non-GLP immune activation study?**
  + Priming immune response during treatment with a CPI product
  + Vaccination to increase overall activation status and therefore expression of CPI ligand
  + Assess immune function in relevant cell populations – based on pattern of expression of CP marker and its ligand – identify target cells
  + Assess pathology in target tissues to identify any potential AI disease
  + Consider combination of multiple CPI products
  + Compare to existing data set generated with licensed products in combination

+ **Caution around over-predicting adverse effects**
  + Can be managed in the clinic if required

Caution around over-predicting adverse effects
  + Can be managed in the clinic if required
Translation to the clinic

+ Consider immune status of clinical population
  + Pre- or post-chemotherapy
  + Intact or depleted/suppressed immune system
  + Concurrent treatments
Summary

+ The immune system is a complex interaction of multiple cell populations and control mechanisms which provide sufficient sensitivity to identify and eliminate pathogens, while limiting more extensive damage

+ We have the potential to adjust the extent of an immune response by removing elements of the peripheral tolerance system
  + Effectively taking the brake off the immune response and amplifying magnitude/duration of a response
  + Increases tumour surveillance, but also has potential to increase anti-self responses, leading to autoimmune disease

+ Important to understand the magnitude and downstream effects of any increases in immune activation when designing non-clinical safety assessment packages
  + Comparative immune function
  + Measure functional responses induced in non-clinical models before proceeding to the clinic
Summary

+ Consider relative immune status of non-clinical animal and subsequent clinical population
+ Consider the effects that may be mediated by combinations of CPI products
  + Redundancy is built into the immune system; subverting this when multiple pathways are affected
+ Compare findings with existing products and combinations
  + Both non-clinically and clinically
+ Understand the pathways that are being blocked and their relative contributions to peripheral tolerance
  + CTLA-4 is constitutively expressed on T_{reg} – block it and you block their function
  + Other markers linked with T cell activation – blockage may lead to enhanced cell activation phase, which can be eventually be brought under control by other mechanisms such as T_{reg}
  + Effects of combined blocking???
+ Biology, biology and more biology!
Thank you for your time at SOT 2018 San Antonio

Visit us at booth #839

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